Chem. Soc. **120**:9787-9792 (1998)) will lead to a film with a greater free volume as compared with a film formed from the same 12mer directly bound to the surface.

While the surface density of single-stranded $SA_{20}12F$ strands (15 ± 4 pmol/cm^2) was lower than that of S12F (34 ± 1 pmol/cm^2), the particles modified with a 32-mer using the identical surface modification showed comparable stability compared to those modified with 12-mer. As anticipated, the hybridization efficiency of the $SA_{20}12F/12F'$ system (6.6 ± 0.2 pmol/cm^2, 44%) was increased to approximately 10 times that of the original S12F/12F' system, Table 7.

M. Effect of Electrolyte Concentration During Oligonucleotide Attachment

In working with the S12F sequence a salt aging step was found to be crucial in obtaining stable oligonucleotide modified nanoparticles (see Example 3). The gold nanoparticles modified with S12F in pure water fused together irreversibly to form a black precipitate upon centrifugation, while those aged in salt resisted aggregation when centrifuged, even in high ionic strength solutions. It is proposed that the increased stability is due to higher oligonucleotide surface coverages which leads to greater steric and electrostatic protection. Using the $SA_{20}12F$ modified particles, the effect of electrolyte conditions on oligonucleotide surface loading was investigated. As shown in Table 8, final surface coverages for gold nanoparticles which were exposed to oligonucleotides in water for 48 hours are much lower (7.9 ± 0.2 pmol/cm^2) compared to those that were 'aged' in salt, or prepared by increasing the salt concentration gradually over the course of the final 24 hours of the experiment (see above).

It is important to note that gold nanoparticles as synthesized irreversibly agglomerate even in very low ionic strength media. Indeed, they are naturally incompatible with salts and especially polyanions such as oligonucleotides. This aging treatment is essential for preparing stable oligonucleotide particles. Therefore, the particles must be initially modified with alkylthiol oligonucleotides in water prior to gradually increasing the ionic strength. It is likely that oligonucleotides initially lie flat, bound through weak interactions of the nitrogenous bases with gold. A similar mode of interaction has been proposed for

oligonucleotides on thin films (Herne et al., *J. Am. Chem. Soc.* **119**:8916-8920 (1997)). However, the interaction between oligonucleotides and the positively charged nanoparticle surface (Weitz et al., *Surf. Sci.* **158**:147-164 (1985)) is expected to be even stronger. In the aging step, the high ionic strength medium effectively screens charge repulsion between neighboring oligonucleotides, as well as, attraction between the polyanionic oligonucleotide and the positively charged gold surface. This allows more oligonucleotides to bind to the nanoparticle surface, thereby increasing oligonucleotide surface coverage.

N. Effect of Oligonucleotide Spacer Sequence on Surface Coverage.

In order to examine how the sequence of the spacer affects oligonucleotide coverage on Au nanoparticles, fluorescein-modified 32-mer strands, with 20 dA and 20 dT spacers inserted between a 3' propylthiol and the fluorescein-labeled 12-mer sequence, were prepared. The most notable result of surface coverage and hybridization studies of nanoparticles modified with S3'T_{20}12F and S3'A_{20}12F is the greater surface coverage achieved with the 20 dT spacer (35 ± 1 pmol/cm^2), in comparison to the 20 dA spacer (24 ± 1 pmol/cm^2). The number of hybridized strands was comparable, although the percentage of surface bound strands which hybridized was lower for ST_{20}12mer nanoparticles (79 %) than the SA_{20}12 nanoparticles (~94%). These results suggest that dT rich oligonucleotide strands interact non-specifically with the nanoparticle surface to a lesser degree than dA rich oligonucleotide strands. Consequently, 20dT spacer segments may extend perpendicular from the gold surface, promoting higher surface coverages, while 20dA spacer segments block gold sites by lying flat on the particle surface.

O. Effect of Coadsorbed Diluent Oligonucleotides

In addition to efficient hybridization, another important property of oligonucleotide modified nanoparticles is the possibility of adjusting the total number of hybridization events. This is most readily accomplished by adjusting the surface density of recognition strands. Other researchers have used coadsorbed diluent alkylthiols such as mercaptohexanol with modified oligonucleotides on gold electrodes to control hybridization (Steel et al., *Anal. Chem.* **70**:4670-4677 (1998); Herne et al., *J. Am. Chem. Soc.* **119**:8916-8920 (1997)).

However, the inherent low stability of unprotected gold nanoparticles poses serious constraints on the choice of diluent molecule. A thiol modified 20 dA sequence (SA_{20}) [SEQ ID NO:55] proved to be suitable in terms of maintaining particle stability in the high ionic strength buffers which are needed for hybridization and protecting the surface from non-specific adsorption.

Nanoparticles were modified using solutions containing different recognition strand (SA_{20}12F) to diluent (SA_{20}) strand molar ratios. The resulting particles were analyzed by the fluorescence method described above to determine the SA_{20}12F surface density, and then tested for hybridization efficiency with 12'F.

The SA_{20}12F surface density increased linearly with respect to the proportion of SA_{20}12F to SA_{20} in the deposition solution, Figure 30. This is an interesting result because it suggests that the ratio of SA_{20}12F to SA_{20} attached to the nanoparticles reflects that of the solution. This result is in contrast to what is normally seen for mixtures of short chain alkyl or ω-functionalized thiols, where solubility and chain length play a crucial role in adsorption kinetics (Bain et al., *J. Am. Chem. Soc.* **111**:7155-7164 (1989); Bain et al., *J. Am. Chem. Soc.* **111**:7164-7175 (1989)).

The amount of complementary 12F' oligonucleotide which hybridized to each different sample also increased linearly with increasing SA_{20}12F surface coverage, Figure 31. The fact that this relationship is well defined indicates that it is possible to predict and control the extent of hybridization of the nanoparticle-oligonucleotide conjugates. This suggests that hybridization of 12F' becomes more difficult at higher SA_{20}12F coverages, which is most likely a result of steric crowding and electrostatic repulsion between oligonucleotides.

P. Summary.

This study has shown that it is important to achieve a balance between oligonucleotide coverage high enough to stabilize the nanoparticles to which they are attached, yet low enough so that a high percentage of the strands are accessible for hybridization with oligonucleotides in solution. This has been achieved by adjusting salt